REMARKS

In the Office Action of July 17, 2003, the Examiner objected to several informalities in the specification as filed on March 6, 2000. Applicants have amended the specification to overcome these objections. No new matter has been added. The Examiner objected to the form and dependencies of several claims. Applicants have amended the relevant claims to overcome these objections, including explicitly reciting hybridization in claims 80 and 93.

In the Office Action of July 17, 2003, the Examiner objected to the specification as apparently improperly incorporating by reference various documents and cited in support *Advanced Display Systems Inc. v. Kent State University*, 212 F.3d 1272, 54 USPQ2d 1673 (Fed. Cir. 2000). However, the Examiner didn't show why the present application didn't properly incorporate by reference the various cited (and incorporated) patents and publications. Just the opposite, the present application clearly identified with particularity what specific material it incorporated, as understood by a person of ordinary skill in the art. This is the standard stated, for example, in *Quaker City Gear Works, Inc. v. Skil Corp.*, 747 F.2d 1446, 223 USPQ 1161 (Fed. Cir. 1984) and and also adopted in *Advanced Display Systems Inc. v. Kent State University*. A person of ordinary skill in the art (i.e., a Ph.D. in the present case, according to the Examiner) is clearly able to use, in any publication, the table of contents and the index, or read any issued patent.

In *Advanced Display Systems* the Court dealt with incorporation by reference to determine anticipation. *In re Lund*, 376 F.2d 982, 991, 153 USPQ 625, 633 (CCPA 1967), the Applicant's filing date preceded the issue date of the patent reference. The abandoned application contained subject matter which was essential to the rejection but which was not carried over into the continuation-in-part application. The court held that the subject matter of the abandoned application was not available to the public as of either the parent's or the child's filing dates and thus could not be relied on in the rejection under 35 USC §102(e). Thus, the *In re Lund* case in not applicable to the present application.

In short, a Ph.D. is clearly able to read the table of contents and the index to in any publication incorporated by reference (which incorporated publications were available to the public before the filing date of this application. Furthermore, a Ph.D. is clearly able to read any issued patent incorporated by reference into this application. Regarding the printed publications, should the Examiner require, Applicants will bring over into the pending specification the relevant text portions.

In the Office Action of July 17, 2003, the Examiner rejected claims 80-108 under 35 USC §112, first paragraph, as failing to comply with the enablement requirement. Applicants respectfully disagree with these rejections and show why the Examiner did not meet the prima facie burden of 35 USC §112, first paragraph. Furthermore, as explained in detail below and pointed out specifically with respect to the original specification, Applicants fully complied with the enablement requirement.

Applicants respectfully submit that the Examiner, when rejecting claims 80-108 under 35 USC §112, first paragraph, did not comply with the prima facie requirement to make such rejections. Applicants believe that the Examiner made several unsupported statements that are not sufficient to make and support rejections under 35 USC §112, first paragraph. Furthermore, Applicants believe that the Examiner may have misunderstood the subject matter of the present method claims when citing the case law since the cited case law deals with different types of claims, as explained below. Applicants also respectfully submit that the Examiner made several contradicting statements with respect to the issued US patents 5,200,313; 5,230, 866; and 6,077,674 (which are presumed to be valid and thus their specifications are presumed to provide enabling description). Applicants also respectfully submit that the cited case law is inapplicable to the present application, as we explain in detail below.

To support the above rejection, the Examiner cited *Enzo Biochem Inc., v. Calgene, Inc.* 188 F.3d 1362, 52 USPQ.2d (BNA) 1129 (Fed. Cir. 1999), but the *Enzo* case is fundamentally different and is **inapplicable to the present patent application.** The *Enzo Biochem* case is inapplicable to the present patent application because there the patentee tried to prove enablement by using post-filing evidence. In *Enzo Biochem*, the Court determined that US Patents 5,190,931 and 5,208149 in suit provided only a

basic blueprint, "the mere germ of the idea," for exploiting the technology in eukaryotes. On the other hand, the present specification provides a detailed teaching that satisfies the enablement requirement, as explained below.

Specifically, in the patents licensed by *Enzo Biochem*, during the patent prosecution, the patentee provided a Declaration by Saul Silverstein justifying the enablement by citing examples of post filing success including citing numerous references published after the filing date. The Silverstein Declaration asserted that the successful results obtained in the cited publications were because the authors followed the teachings provided in the patent. The court put little weight on such extrinsic evidence. Therefore, *Enzo Biochem Inc., v. Calgene, Inc.*, as used by the Examiner, is **not applicable** to the pending claims. We do not rely on any post filing activity but only on the pending specification. The specification provides a detailed enabling disclosure for all pending patent claims, as is shown in detail below.

In paragraph 11 of the Office Action, the Examiner stated: "Independent claims 80, 93, 106, and 107 are all drawn to '[a] method of analyzing a sample.' Each of said claims require the detection and evaluation of a signal through the use of confocal microscopy, and in the case of claims 80 and 93, hybridization is to have taken place prior to the signal being detected and evaluated. In accordance with the recited method steps, and using claim 80 as an example for the remaining independent claims, one is to perform two method steps prior to performing said confocal microscopy."

Applicants amended claims 80 and 93 to positively recite hybridization, which was previously, perhaps, indirectly recited in these claims. However, claims 106 and 107 do not require confocal microscopy. Claims106 and 107 are directed to a method of analyzing a sample in a microfluidic device where hybridization doesn't necessarily have to be performed.

In paragraph 12 of the Office Action, the Examiner rejected the pending claims under 35 USC §112, first paragraph, mentioned three examples provided in the specification, and stated: "None of these example disclose starting materials and reaction conditions where a first reaction chamber and second reaction chamber are

used to perform the defined reactions, and to then perform confocal microscopy on a hybridized sample and then make any analysis of the results."

Applicants respectfully disagree with these statements since Applicants provided an enabling description. Specifically, Applicants incorporated by reference Molecular Cloning Techniques: A Laboratory Manual (Sambrook, J., Fritsch, E.F. and Maniatis, T.) Cold Spring Harbor Laboratory Press, 1989; and PCR Protocols: A Guide to Methods and Applications (Innis, M., Gekfand, D., Sninsky, J. and White, T., eds.) Academic Press, 1990. A person of ordinary skill in the art (i.e., a PhD as acknowledged by the Examiner) is clearly able to read these reference manuals to determine starting materials and reaction conditions to perform the claimed methods.

Specifically, Chapters 7, 9, and 10 of Molecular Cloning are directed to preparative reactions, that is, sample extraction, PCR amplification, extraction of intracellular material, nucleic acid fragmentation, labeling, extension reactions and transcription reactions. Chapters 6 and 11 of Molecular Cloning are directed to analysis reactions including size based analysis (microcapillary electrophoresis) or sequence based analysis (hybridization). Chapters 2, 3, 4, 7, and 9 of Molecular Cloning are directed to DNA extraction including denaturing of contaminating (DNA binding) proteins, purification, filtration or desalting. Chapter 14 of Molecular Cloning is directed to amplification by performing PCR, LCR, 3SR, NASBA. Chapter 10 of Molecular Cloning is directed to IV transcription, and Labeling by incorporating label into amplified or transcribed sequence, labeling primers, incorporation of labeled dNTPs, covalent attachment of a particular detectable group. Furthermore, the above processes are described in numerous US patents incorporated by reference. For example, PCR amplification is described in several patents incorporated by reference such as US Patent 4,965,188 to Mullis or US Patent 5,304,487 to Wilding, cited by the Examiner. (If US Patent 5,304,487 doesn't provide an enabling description then it cannot be cited as a prior art reference against the present application.)

The Examiner also rejected the pending claims under 35 USC §112, first paragraph, stating that "[t]he situation at hand is analogous to that in *Genentech v. Novo Nordisk A/S* 42 USPQ2d 1001." Applicants respectfully disagree with the

Examiner's statements because *Genentech v. Novo Nordisk,* as applied by the Examiner is again **not applicable** to the pending claims.

In *Genentech v. Novo Nordisk of America Inc.,* 108 F.3d 1361, 42 USPQ2d 1001 42 USPQ2d 1001 (Fed. Cir. 1997) the invention at issue involved a method of producing a protein consisting essentially of human growth hormone. (*Id.* at 1363) The specification of the patent in suit did not describe in any detail how to make the hormone using the stated process. Furthermore, the specification did not describe the reaction conditions required to produce the hormone. (*Id.* at 1365) Thus, the Court found that undue experimentation was required.

On the contrary, the pending claims are not directed to a method of producing a protein or producing a gene or any specific biological polymer, but are directed to a method of analyzing a sample in an integrated microfluidic device. That is, the present claims are directed to using a specific integrated microfluidic device for performing one, two or more processes. Therefore, *Genentech v. Novo Nordisk,* as applied by the Examiner, is **not applicable** to the pending claims. (Moreover, the present specification provides a detailed enabling disclosure for the claimed processes, as explained above. Furthermore, the Examiner cited *Amgen, Inc. v. Chugai Pharm. Co., Ltd.,* 927 F.2d 1200, 1213, 18 USPQ2d 1016, 1027 (Fed. Cir. 1991), which itself noted that the *Wands* factors are not mandatory and the corresponding enablement inquiry depends on the facts. The types of claims pending in the present application are very different than the claims at issue in the cases cited.)

Applicants do not dispute that the Federal Circuit has required a heightened standard of enablement due to the unpredictability of the biotechnology art, but this standard is applied in view of the claimed subject matter. The Federal Circuit had rejected Applicant's claim as not enabled due to the unpredictability of the microbiology of bacteria, as in *In re Vaeck* 947 F.2d 488, 496, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991), or has not sufficiently described the method of producing a protein, as in *Genentech v. Novo Nordisk of America Inc.,* 108 F.3d 1361, 42 USPQ2d 1001 42 USPQ2d 1001 (Fed. Cir. 1997). However, the present claims are not directed to any such subject matter. As explained above, the pending claims are directed to using an integrated

microfluidic device, and a method of analyzing a sample in an integrated microfluidic device. The present specification provides detailed enabling disclosure of such use, and of all the claimed processes.

The Examiner also cited US Patent 5,230,866, for referring to "numerous difficulties associated with junctions in a device intended for fluid manipulation." Applicants respectfully disagree with the Examiner's statements, and even believe that these statements are contradictory. Initially, US Patent 5,230,866 (the '866 patent) described a "number of factors contribute to the instability of the junction," but the listed difficulties (cited by the Examiner) existed in 1991 prior to the description provided in the '866 patent. That is, the '866 patent also provides a written description of how to overcome the mentioned problems and make an analytical cartridge.

Specifically, the '866 patent claims an analytical cartridge with a housing containing vent passageways, capillary passageways, a non-capillary vent-surge chamber, etc. The application, issuing as the '866 patent, was filed in 1991 and the patent issued on July 27, 1993. The difficulties (cited by the Examiner) existed in 1991 and were resolved in the enabling written description, provided in the '866 patent (by the virtue of presumption of validity of this issued patent). The Examiner didn't provide any proof that the '866 patent is invalid. Therefore, on July 27, 1993, a person of ordinary skill in the art learned about the enabling solutions provided in the '866 patent. In other words, the solution provided in the '866 patent was publicly known and thus no longer an unsolvable problem to a person of ordinary skill in the art (for two years before the earliest priority date of the present application). Therefore, the Examiner's statements are contradictory (unless the Examiner can prove insufficient written description, including a non-enabling description, provided by the '866 patent, in which case the listed problems could remain, but the '866 patent would be invalid of course). Therefore the Examiner's statements in connection with the '866 patent are contradictory and inapplicable to the pending claims.

The Examiner also cited statements from US Patent 6,077,674 to Schleifer et al., directed to a method of making full-length oligonucleotide arrays. (Applicants by no means admit the correctness or validity of the statements made in US Patent 6,077,674,

i.e., the '674 patent) . The '674 patent, states that "in [the] procedure described by Fodor et al. from Affymetrix U.S. Pat. No. 5,405,783, a photo-deprotection step is used where the protecting group on the phosphoramidite is removed by exposing a photosensitive protecting group to light. Four photo masks are used to create patterns to de-protect areas of the substrate and then a nucleotide is added to these regions. This technique requires four masks for each layer of nucleotides. While this technique allows for the production of high-density oligonucleotide arrays, it is less efficient than traditional phosphoramidite synthesis chemistry. With efficiencies of about 90 to 95 percent, the percentage of full-length oligonucleotides within a feature is further reduced to about 9 to 27 percent for oligonucleotides that are 25 nucleotides long (0.90^{25} to 0.95^{25})." That is, even Schleifler doesn't dispute that Fodor provides enabling teaching. In any case, the present claims are not directed to methods of making full-length oligonucleotide arrays, but to methods of analyzing a sample in an integrated microfluidic device. Thus, the Examiner's statement does not support the rejections under 35 USC §112, first paragraph.

In the context of 35 USC §112, first paragraph, the Examiner also cited statements from US Patent 5,200,313 to Carrico, and mentioned that this patent "similarly identifies problematic aspects of hybridization reactions." On pages 10 and 11, the Examiner verbatim listed points 1-6 discussed in US Patent 5,200,313 (the '313 patent). However, the '313 patent claims a nucleic acid hybridization method for determining a polynucleotide having a particular base sequence. The application, issuing as the '313 patent, was filed in 1988 (when perhaps the listed problematic aspects were applicable) and the '313 patent issued on April 23, 1993. The '313 patent has a presumption of validity, including providing an enabling disclosure. That is, the patent specification discloses how to resolve the issues related to the extent and specificity of hybridization, i.e., the issues in points 1 – 6. Therefore, on April 23, 1993, a person of ordinary skill in the art learned about the enabling solutions provided in the '313 patent, which patent claims nucleic acid hybridization methods for determining a polynucleotide. That is, the solution provided in the '313 patent was publicly known more than two years before the earliest priority date of the present application. Therefore, the Examiner's statements are contradictory with respect to the '313 patent,

and do not support rejections under 35 USC §112, first paragraph (unless the Examiner can overcome the presumption of validity of the '313 patent and can prove insufficient written description, including non-enabling description provided in the '313 patent).

For the above reasons, the Examiner did not meet the prima facie burden for rejecting claims 80-108 under 35 USC §112, first paragraph. Furthermore, the present application clearly satisfies the written description and the enablement requirements and therefore the rejections under 35 USC §112, first paragraph, should be withdrawn.

The Examiner rejected claims 80-108 under 35 U.S.C. 112, second paragraph, as failing to set forth the subject matter regarded as the invention. Applicants respectfully disagree with this rejection.

Applicants respectfully point out that methods were indeed considered part of the present invention, as evidenced, for example, by the statement provided in the original specification on page 5, lines 20-26: "The device of the invention is generally capable of performing one or more sample acquisition and preparation operations, in combination with one or more sample analysis operations. For example, the device can integrate several or all of the operations involved in sample acquisition and storage, sample preparation and sample analysis, within a single integrated unit. The device is useful in a variety of applications, and most notably, nucleic acid based diagnostic applications and de novo sequencing applications."

Therefore, the above clearly identifies that the invention includes a method of analyzing a sample in a microfluidic device, and such methods are claimed in the present application. Separately, Applicants currently amended the title of the application to show that the above methods are being claimed. Accordingly, Applicants respectfully ask the Examiner to withdraw the rejections under 35 U.S.C. 112, second paragraph.

The Examiner rejected claims 80-110, 112-114, and 116-120 under 35 U.S.C. §103(a) as obvious US Patent 5,304,487 to Wilding et al. in view of Staecker et al., and US Patent 5,587,128 to Wilding et al. The Examiner also rejected claims 111 and 115 under 35 U.S.C. 103(a) as being unpatentable over US Patent 5,304,487 to Wilding et

al. and US Patent 5,587,128 to Wilding et al. and Staecker et al., as applied to claims 80-110, 112-114, and 116-124, and further in view of US Patent 5,1227,730 to Brelje et al. Applicants respectfully disagree with these rejections for the following reasons:

As claimed in claim 80, for example, the present invention is a method of analyzing a sample in an integrated microfluidic device having at least two chambers in fluid communication. The method includes supplying the sample into a first chamber, performing a first reaction in the first chamber, moving the sample from the first chamber to the second chamber, and performing a second reaction in the second chamber, wherein the second reaction is different from the first reaction, and wherein the first or the second chambers are selected from the group of chambers adapted to perform a preparative reaction, an analysis reaction including hybridization, sample acquisition, DNA extraction, amplification, IV transcription or labeling. The method also includes performing confocal microscopy on the hybridized sample by detecting an optical signal from the hybridized sample inside of the chamber using a reader device located outside of the chamber; receiving a signal output from the reader device; and analyzing the signal output with a digital computer to indicate a property of the sample based on the confocal microscopy.

As further claimed in claim 110, for example, the microfluidic device includes a probe array immobilized on an internal surface and the recited confocal microscopy is used to detect the hybridized sample on the probe array.

As claimed in claim 106, the method of analyzing a sample in an integrated microfluidic device includes supplying the sample into a first chamber selected from the group consisting of a chamber adapted to perform a preparative reaction, an analysis reaction, sample acquisition, DNA extraction, amplification, IV transcription or labeling. The method also includes moving the sample from the first chamber to a second chamber by employing a valve located in a channel between the first chamber and the second chamber, the second chamber being selected from the group consisting of a chamber adapted to perform a preparative reaction, an analysis reaction, sample acquisition, DNA extraction, amplification, IV transcription or labeling; and receiving a signal output from a reader device and indicating a property of the sample.

The Examiner made **three-reference obviousness** rejections and **four-reference obviousness** rejections. Applicants respectfully submit that, when making these rejections, the Examiner did not establish the *prima facie* case of obviousness as explained in detail below:

Obviousness under 35 U.S.C. §103

The Federal Circuit has restated numerous times the criteria for rejecting a claim under 35 U.S.C. §103 as being obvious. For example, as stated in In re Fritch:

> In proceedings before the Patent and Trademark Office, the Examiner bears the burden of establishing a prima facie case of obviousness based upon the prior art. *In re Piasecki*, 745 F.2d 1468, 1471-72, 223 USPQ 785, 787-88 (Fed. Cir. 1984). A[The Examiner] can satisfy this burden only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references. *In re Fine*, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988) (citing *In re Lalu*, 747 F.2d 703, 705, 223 USPQ 1257, 1258 (Fed. Cir. 1988)). The patent applicant may then attack the Examiner's prima facie determination as improperly made out, or the applicant may present objective evidence tending to support a conclusion of nonobviousness. *In re Heldt*, 433 F.2d 808, 811, 167 USPQ 676, 678 (CCPA 1970).
> In re Fritch, 23 USPQ2d 1780, 1783 (Fed. Cir. 1992)

Obviousness cannot be established by combing the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. Under section 103, teachings of references can be combined *only* if there is some suggestion or incentive to do so. *ACS Hosp. Systems, Inc. v. Montefiore Hosp.*, 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)

> Although couched in terms of combining teachings found in the prior art, the same inquiry must be carried out in the context of a purported obvious modification of the prior art. The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggested the desirability of the modification. *In re Gordon*, 733 F.2d at 902, 221 USPQ at 1127.
> In re Fritch, 23 USPQ2d 1780, 1783 (Fed. Cir. 1992), emphasis ours.

Furthermore, the MPEP provides the following requirements on the Examiner to establish the *prima facie* case of obviousness:

> **2143 Basic Requirements of a Prima Facie Case of Obviousness [R-1]**

To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. MPEP '2143

In the Office Action of June 13, the Examiner stated:

> 7. Wilding 1 discloses a method for analyzing a sample in an integrated microfluidic device that has a plurality of chambers that are in fluid communication with each other. As seen in column 2, the diameter of the channels can range from 0.1 μm to 500 μm. Said channels are in communication with "fluid handling regions." Said regions are considered to meet the limitation of applicants "at least two chambers." …
>
> …
>
> 8. Column 4, first paragraph, teaches explicitly of the optional use of valves within the fluid communication means.

Applicants respectfully disagree with these statements by the Examiner.

While it is true that the two patents to Wilding teach extraction, purification and a subsequent amplification reaction of the same, these are not performed in two separate chambers separated by a valve. The teaching in US Patent 5,304,487 directed to several channels providing communication for different fluid hanfling regions is fundamentally different than the claimed processing in at least two chambers separated by a valve, as recited in claims 106, 107, 120 or 124.

In US Patent 5,587,128, for example in col. 12, Wilding mentions reaction chambers and flow channels of different sizes and cross-sections only generically without specificity. Furthermore, US Patent 5,587,128 describes analytical devices shown in Figs. 6 through 13. For example, in connection with Fig. 7 (and similarly Fig. 10), Wilding teaches in col. 21, lines 51 – 59 as follows: "FIG. 7 shows a schematic plan view of a substrate 14 fabricated with a system of flow channels 40 connected via channel 20 to ports 16 and a reaction chamber comprising sections 22A and 22B separated by a flow path 20B. The presence of amplified polynucleotide product in a sample will influence the flow characteristics within the flow channels. The channels 40 in this embodiment are symmetrically disposed and have a progressively narrower diameter towards the center of the pattern." (Emphasis ours) In some places Wilding

refers to two reaction chambers (instead of sections 22A and 22B), but still there is no valve located in the flow path between the chambers.

US Patent 5,304,487 describes very similar analytical devices as the 128 patent, suggesting or having a valve only at the input or output ports, but not between the reaction chambers. The Examiner referred to US Patent 5,304,487, col. 4, first paragraph, that recites:

> In one embodiment, the detection region may comprise binding moieties, capable of binding to the analyte to be detected, thereby to enhance and facilitate detection. The detection region also may comprise a fractal region, i.e., a region of serially bifurcating flow channels, sensitive to changes in flow properties of a fluid sample, as is disclosed in U.S. Ser. No. 07/877,701, filed May 1, 1992, the disclosure of which is incorporated herein by reference. The device also may be fabricated with at least three inlet ports, in fluid communication with the flow system, provided with valves, e.g., in an appliance used in combination with the device, for closing and opening the ports to enable the control of fluid flow through the mesoscale flow system.
>
> The mesoscale devices can be adapted to perform a wide range of biological tests. Some of the features and benefits of the devices are summarized in Table 1. A device may include two or more separated flow systems, e.g., fed by a common inlet port, with different cell handling chambers in each of the systems to enable two or more analyses to be conducted simultaneously. The devices can be utilized to implement a range of rapid tests, e.g., to detect the presence of a cellular or intracellular component of a fluid sample. The devices may be utilized to detect, e.g., a pathogenic bacteria or virus, or for cell sorting. The invention provides methods and devices for a wide range of possible analysis. Assays may be completed rapidly, and at the conclusion of the assay the chip can be discarded, which advantageously prevents contamination between samples, entombs potentially hazardous materials, and provides inexpensive, microsample analyses.
>
> (Col. 4, lines 7 – 40, Emphasis ours)

Therefore, Wilding only teaches the use of a valve in the appliance device or at the input (output) port to his mesoscale system. On the other hand, for example, claim 106 (or claim 107) recites moving the sample from the first chamber to the second chamber by employing **a valve** located in the channel between the first chamber and the second chamber. In US Patent 5,587,128 or US Patent 5,304,487, Wilding not only does not teach the use of two separate chambers separated by a valve (including the other limitations recited in claim 106 or claim 107), Wilding also does not even hint about the use of a valve located in the flow channel between the chambers. Wilding only uses a

valve at the entrance into his device. The publication of Staecker et al. alone or in combination with U.S Patent 5,1227,730 to Brelje et al. does not disclose the method claimed in independent claim 106 or 107 using a valve located in the channel between the first chamber and the second chamber. Therefore, independent claim 106 and independent claim 107 are clearly patentable over prior art of record.

Applicants respectfully disagree with some of these statements by the Examiner. While it is true that US Patent 5,304,487 and US Patent 5,587,128 together suggest detection of a signal through a transparent cover, they do not disclose or suggest the claimed "inside chamber" use of confocal microscopy when combined with the publication Staecker et al. The Examiner didn't provide any evidence that this would be obvious.

Applicants respectfully submit that the three-reference combination of the two Wilding patents and the publication Staecker et al. do not disclose all limitations of independent claim 80 or independent claim 93. Furthermore, this three-reference combination does not disclose detection of a hybridized sample on a probe array using confocal microscopy, as recited in dependent claim 110 or in dependent claim 114.

As admitted by the Examiner, US Patent 5,304,487 and US Patent 5,587,128 do not disclose or suggest the use of confocal microscopy. There is no teaching in the publication Staecker that would disclose or even suggest the use of confocal microscopy for detecting an optical signal from the hybridized sample inside of the chamber using a device located outside of the chamber. Staecker only teaches the use of confocal microscopy on a slide.

Specifically, on page 76, Staecker discloses the following:

INTRODUCTION

The nerve growth factor (NGF) family of neurotrophins has been found to play an important role in the innervation of the inner ear of the mouse (4,6); however, thus far a source of NGF production has not been localized in the tissues of the inner ear (6). Attempts to localize NGF production using *in situ* hybridization have been unsuccessful (6) presumably because of the very low copy number of NGF mRNA in this system. We have detected the presence of NGF mRNA in the otocyst-cochleovestibular

ganglion complex by RT-PCR and have attempted to apply this technique to localize sites of NGF mRNA expression in the inner ear.

By modifying the method of *in situ* polymerase chain reaction (PCR) on tissue preparations as described by Nuovo (5), we have produced a pool of NGF cDNA by reverse transcription (RT) of mRNA in tissue sections mounted on microscope slides. Following this, PCR amplification of the tissue section with addition of a fifth, fluorescent-labeled deoxynucleotide and a set of NGF-specific primers, allows identification of cells containing NGF cDNA by fluorescent microscopy. The advantages of this method are 1) minute amounts of mRNA can be detected and localized at the cellular level, 2) image analysis of confocal microscopic images allows analysis and a rough estimation of accumulated labeled cDNA and 3) time-consuming autoradiography is avoided. (Emphasis ours)

Thus, Staecker discloses only in situ hybridization and in situ polymerase chain reaction (PCR) but not in situ confocal microscopy; this is confirmed by the following teaching of Staecker. On page 78, Staecker discloses:

After placing the PCR mixture on the specimens, plastic coverslips were placed over the specimen and the surrounding nail polish ring. The slides were placed on the PCR machine block (coy slide cycler, prototype model; Grass Lake, MI, USA). The PCR process was initiated with a time-delay file of 8 min at 82°C. Once the block temperature reached 75°C, the coverslips were lifted and 3 µl of *Taq* buffer containing PCR mixture were added to the slide was covered with mineral oil that had been preheated to 80°C in a separate dry block. Twenty cycles of amplification were run with the following program: an initial denaturing step of 94°C x 2 min, linked to a cycling file of 1) 55°C x 1 min, annealing segment; 2) 72°C x 1 min, extend segment; 3) 92°C x 1 min, denaturing segment; then repeat the cycle of steps 1-3, etc. After the completion of 20 cycles, slides were removed and placed in xylene to remove the mineral oil. The xylene was removed, and the tissue sections were washed 2 times for 20 min each in 0.1 x standard saline citrate (SSC) at 45°C to remove excess fluorescent nucleotides.

ANALYSIS

Slides were examined with either a Zeiss axiophot fluorescent microscope (Carl Zeiss, Thornwood, NY, USA) (450-490-nm wave length fluorescent epilumination) or a Bio-Rad MRC 600 Confocal microscope (Hercules, CA, USA) (Image Analysis Facility, Albert Einstein College of Medicine). Data collection was carried out using a 40x lens and 3x Kallman sampling. The confocal's histogram function was used to compare relative fluorescence per pixel of image, allowing determination of fluorescent staining intensity.

Therefore, Staecker does not disclose or even suggest the use of confocal microscopy for detecting an optical signal from the hybridized sample inside a chamber using a device located outside of the chamber. Moreover, Staecker **teaches away** from this "in situ" use of confocal microscopy. Specifically, after hybridization, Staecker teaches taking the slides out, and then performing confocal microscopy. In other words, Staecker does not teach (or even suggest) performing confocal microscopy on the hybridized sample by detecting an optical signal from the hybridized sample inside of the chamber by a confocal microscope located externally to the chamber. Importantly, none of these references provides any motivation to combine US Patents 5,304,487 and 5,587,128 together with the publication Staecker.

Furthermore, US Patents 5,304,487 and 5,587,128 together with the publication Staecker do not disclose or even suggest the detection of a hybridized sample on a probe array using confocal microscopy, as recited in dependent claim 110 or in dependent claim 114.

While in U.S Patent 5,1227,730 Brelje discloses a scanning confocal imaging system, Brelje does not teach (or even suggest) performing scanning confocal microscopy on the hybridized sample by detecting an optical signal from the hybridized sample inside of a chamber by a confocal microscope located externally to the chamber. There are different requirements and requirements for performing confocal microscopy on a slide and for performing confocal microscopy on a sample inside of a chamber. (Furthermore, Brelje does not teach (or even suggest) performing the scanning confocal microscopy on the hybridized sample on a probe array.)

Applicants respectfully submit that, when making the above-mentioned rejections, the Examiner did not establish the *prima facie* case of obviousness. The Examiner did not provide any evidence required to show at least some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to combine the mesoscale systems of Wilding et al (described in US Patents 5,304,487 and 5,587,128) together with the publication Staecker directed to histological specimens on a slide. Applicants believe that the two devices (i.e., the mesoscale system and a slice support) are not compatible.

Furthermore, there is not teaching or suggestion to modify, as required to establish the *prima facie* case of obviousness.

Accordingly, independent claims 80, 93,106 and 107 are clearly patentable over the prior art cited by the Examiner. Dependent claims 81 - 92, 94 – 105, and 108 - 125 include additional novel combinations of features. Therefore, all pending claims are in condition for allowance and such action is respectfully requested.

Please any charges or credits to the Deposit Account No. 01-0431.

Respectfully submitted,

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